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THESIS

CHEMICAL PASTEURIZATION OF POULTRY MEAT

Submitted by

Jagdish Singh Teotia

In partial fulfillment of the requirements
for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

August, 1973

COLORADO STATE UNIVERSITY

August, 1973

WE HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER OUR SUPERVISION BY JAGDISH SINGH TEOTIA ENTITLED
CHEMICAL PASTEURIZATION OF POULTRY MEAT BE ACCEPTED
AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY.

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ABSTRACT OF THESIS

CHEMICAL PASTEURIZATION OF POULTRY MEAT

Experiments were conducted to pasteurize poultry skin and meat, utilizing different chemicals, lysozyme, X-irradiation and microwaves. The chemicals tested were lactic acid, acetic acid, sodium carbonate, sodium borate, sodium chloride, potassium hydroxide, chlorine and ethylenediaminetetraacetic acid. Turkey skins and parts or whole carcasses were artificially contaminated with Salmonella senftenberg 775W or Salmonella typhimurium in concentrations ranging between 8×10^3 to 9×10^5 viable cells per ml of contaminating fluid. After each treatment, samples were cultured, plated and tested according to standard methods to determine the susceptibility of Salmonella organisms to the particular treatment.

Water alone destroyed Salmonella organisms at 88, 82 and 77°C, in 15 seconds on skin samples but failed to destroy the test organism at 71°C. Sodium carbonate in a 2 percent solution at pH 11.4 eliminated the test organism at 71°C in 15 seconds on skin samples. A 0.5 percent lactic acid solution at pH 1.7 to 2.1 eliminated the test organism from skin samples at 66 and 71°C in 15 seconds.

Three and four percent lactic acid solutions eliminated Salmonella from turkey drumsticks in 90 minutes at 32°C, whereas a two

percent lactic acid solution failed to destroy the test organism during the same time and temperature conditions. Three percent solutions of acetic acid failed to destroy Salmonella in 90 minutes at 32°C on drumsticks whereas a four percent solution of acetic acid destroyed the test organism in 90 minutes at 32°C. One and 1.5 percent solution of potassium hydroxide eliminated the test organism at 82°C in 60 seconds from drumsticks. Various solutions of sodium carbonate under various conditions inconsistently destroyed Salmonella on drumsticks. A 3400 and 2125 PPM chlorine solution failed to destroy the test organism on turkey drumsticks at 21 ± 2°C in 9 and 24 hours. Ten percent solution of sodium chloride did not eliminate Salmonella from drumsticks in 9 hours at 21 ± 2°C. A 12 percent sodium borate solution destroyed Salmonella and other bacteria on drumsticks in 9 hours at 21 ± 2°C. A combination of sodium borate and chlorine solution did not eliminate Salmonella from turkey drumsticks or turkey tails. All treatments, except the sodium borate, sodium chloride and sodium carbonate solutions, reduced the optical acceptability of the meat.

A 0.1 percent solution of lysozyme reduced Salmonella to an undetected level on drumsticks at 21 ± 2°C within three hours, whereas a 0.5 percent solution of ethylenediaminetetraacetic acid failed to destroy the test organism under the same conditions. Eighty thousand rads of X-ray eliminated the test organism on turkey drumsticks but

failed to remove it from the whole turkey carcass. Microwaves eliminated the Salmonella and other bacteria in 120 seconds from turkey drumsticks and 600 seconds from broiler chicken carcass. None of the treatments changed the appearance of the skin or meat, except that microwaves produced a partially-cooked appearance of the meat.

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CHAPTER I

INTRODUCTION

Salmonellosis is probably the most ubiquitous of the bacterial zoonotic diseases. The largest single reservoir of *Salmonella* in animals has been claimed to exist in domestic fowl, and the sources most frequently incriminated in food borne outbreaks of Salmonellosis in man are poultry and poultry products.

The literature is replete with reports of surveys for *Salmonella* in poultry and meat animals, ranches and feedlots, environs of slaughter, processing plants, processed foods and feed, wild fauna and almost any other environment related to animals and their products. Members of this ubiquitous genus of organisms can apparently be found in the intestinal tract of almost any species in the animal kingdom and therefore in the environment contaminated by animal feces.

Centralization of food processing and operation facilities are common in the United States and Canada and are expected to develop rapidly in other countries. This practice is economical but also provides opportunity for potential health hazards to involve large numbers of people. A single processing plant may distribute products internationally and provide for wide dissemination of food borne disease

agents, whenever improper hygienic practices and control measures occur.

Centralized processing also has the overall effect of depriving the individual consumer of personal selectivity of his food sources and of transferring the responsibility for the wholesomeness and safety of the product to the processor. The processor may or may not accept this trust as inviolate.

Modern technology in poultry industry has made available many new food products that are frozen, canned, or even dried which may be either cooked or raw. Wide distribution of these products and their increased use for mass feeding in cafeterias and large institutions provide a wide avenue for contamination if strict sanitation is not maintained in centralized food processing plants.

It is virtually impossible with present technology to completely exclude pathogenic microorganisms from food. It is possible, however, by means of improved environmental sanitation, time-temperature control, modern processing technology, protective systems of packaging, storing and distribution of food, to reduce the numbers of such organism during processing. The actions necessary to prevent the build up of hazardous microorganism must be applied throughout the many steps required to move food from harvest to consumer.

Any method which might satisfactorily eliminate pathogens from meat is worthy of investigation. Pasteurization of many foods such as

milk, dairy products, beer, wine, pickles, etc. is regular practice now. Heat processing obviously cannot preserve meat in its raw state. When meat products are heated they usually lose their fresh appearance, attractiveness and flavor. The desired degree of bacterial inactivation often results in some overcooking of the meat.

Pasteurization of meat with the help of chemicals, enzymes, or irradiation at low temperature may provide a practical solution to the elimination of pathogens without effecting the quality of the product. Experiments, therefore, were conducted at Colorado State University in the department of Animal Sciences to eliminate *Salmonella* (a potential human pathogen) from poultry meat by utilizing chemicals, an enzyme, and irradiation. Chemicals used in this study were lactic acid, acetic acid, sodium carbonate, potassium hydroxide, ethylenediaminetetraacetic acid, sodium chloride, chlorine, and sodium borate. Lysozyme, X-rays and microwaves were used also to destroy the *Salmonella* organism. *Salmonella senftenberg* 775W and *Salmonella typhimurium* were used as test organisms.

CHAPTER II

REVIEW OF LITERATURE

The widespread distribution of *Salmonella* in animals results in *Salmonella* contamination of human foods derived from animals.

Poultry meats and egg products are often contaminated and have been associated with *Salmonella* outbreaks (25, 27, 35). The wide distribution of *Salmonella* in fresh poultry has been demonstrated in numerous investigations (*Salmonella* Surveillance Report 1964).

Salmonella infections are widespread in poultry. Poultry flocks are commonly infected with one or more species of *Salmonella*. Though these infections are usually of little or no clinical significance, they pose a health hazard to poultry and could produce serious economic loss. Such infections spread rapidly within flocks, as demonstrated by Malhotra et al. (26). They reported *Salmonella* serotypes spread rapidly among chick penmates raised on litter.

The dissemination of *Salmonella* usually starts on the farm and is brought to the processing plant where it is spread to other poultry by contaminated equipment and workers. Morris and Ayers (29) found the incidence of *Salmonella* contamination as high as 9% in samples from turkey processing operations, and as many as 14% of the samples from a chicken processing plant were contaminated. Werner et al.

(39) traced an outbreak of Salmonella typhimurium and found that the source of the organism was ready-to-eat barbecued chicken purchased at a modern supermarket. Wilson et al. (40) reported that a significant portion of all meat purchased in retail markets is contaminated with Salmonella.

A common source of Salmonella organisms in animal populations is animal feed. Some investigators feel that this source is perhaps the most important in terms of overall control of Salmonellosis. In a study of animal feeds in England for the years 1958-1960, Taylor (34) isolated Salmonella serotypes from meat and bone products, mixtures, mashes, fish products, and vegetable products. Salmonella senftenberg 775W was isolated most frequently followed by S. anatum and S. cubana. Salmonella senftenberg 775W has been shown to be the most heat resistant of the Salmonella serotypes and its higher incidence in animal feeds may be due to the fact that most others are destroyed by heat in the processing of these products.

With respect to heat destruction, mostly all Salmonella are readily destroyed at milk pasteurization temperatures. Bayne et al. (6) studied the heat resistance of S. typhimurium and S. senftenberg 775W in chicken meat. Multiple one g samples of meat containing 3×10^8 cells of S. typhimurium Tm-1(#84) after exposure for five minutes to 60°C contained no viable cells. The more resistant S. senftenberg 775W required an exposure of from 10 to 15 minutes at 65°C to kill an equal number of cells.

Ng et al. (30) reported the heat resistance of Salmonella. They studied approximately 300 cultures of Salmonella representing 75 different serotypes and concluded that none were found to be as heat resistant as S. senftenberg 775W. They further reported that strain 775W was more heat sensitive in the lag phase than in the stationary phase of growth. Cells from cultures grown at 44°C were more heat resistant than those grown at either 35 or 15°C. The growth media made no appreciable difference in heat resistance. For any set of growth conditions, strain 775W was always more heat resistant than the other strains of S. senftenberg.

The reported annual incidence of Salmonella in the United States was 10.4 isolations per 100,000 population (2). This incidence is modest due to the large number of unreported cases or erroneously diagnosed illness.

The importance of a routine bacteriological examination of the intestinal tract in avian species was stressed by Faddoul and Fellows (14). An examination of this type may prove to be expensive to the poultry industry but would help in reducing the incidence of Salmonella outbreaks.

Salmonellosis has been cited as one of the most frequent and important causes of food poisoning in humans and has been estimated to cause financial losses of \$10-100 million a year (13). A later report (1) set the annual economic loss from the disease in man at

\$300 million, assuming approximately two million human Salmonellosis cases annually.

Pasteurization of Poultry Meat

Attempts have been made in the past to pasteurize poultry meat and skin with the help of heat, steam, chemicals, and irradiation. Avens (4) tested the hypothesis that the effective turkey carcass pasteurization can be expressed by a linear time vs temperature curve characterized by a point and slope. He did not disprove the hypothesis but failed to establish a pasteurization curve for turkey carcasses using the heat resistant Salmonella senftenberg 775W. The report also showed that flowing steam at $102\pm 2^{\circ}\text{C}$ for 30 minutes apparently contacted all surface areas of the carcass harboring the test organism, but this pasteurization treatment caused partial cooking of carcass skin and meat.

Klose and Bayne (22) reported the surface pasteurization of poultry meat, using condensing vapors, artificially contaminated with Salmonella typhimurium. The pasteurization treatments were studied for their ability to remove natural flora as well as the test organism. Acetone water at 70°C and isopropyl alcohol at 82°C yielded 10,000 to 100,000 fold bacterial reductions in four minute exposures, but trace amounts of these liquids were difficult to remove from the cooked products. Trichloroethylene water vapor at 73°C and water under

reduced pressure at 70°C gave the same order of reduction after eight minute exposure.

Klose et al. (23) further studied the pasteurization of poultry meat by steam under reduced pressure. A reduction of 1000 to 5000 fold in the combined external and internal surface bacterial loads on whole ready-to-cook chicken carcasses was achieved by application of subatmospheric pressure steam at temperatures not exceeding 75°C for four minutes. When carcasses were inoculated with Salmonella typhimurium, limited reductions were obtained by subatmospheric steaming of carcasses inoculated by soaking, while substantial reductions were accomplished on spray inoculated carcasses.

The influence of surface pasteurization and chlorotetracycline on bacterial incidence on fryers was studied by Dawson et al. (9). Their study showed that bacterial counts for fryers pasteurized 5-10 minutes at 135-140°F and chilled in slush ice containing 10PPM, chlorotetracycline were reduced approximately 100 times than on control birds. Wilkinson et al. (41) suggested that an end point temperature of 160°F is sufficient to destroy the low level of pathogenic bacteria which might occasionally be found in turkey rolls after normal processing.

Antibiotics, chlorine, β propiolactone, acids (succinic and citric), and washing has been found quite effective in reducing the Salmonella typhimurium on eviscerated fryer chickens. A significant

reduction in *Salmonella* counts was observed by Thomson et al. (36) by spraying each of the above different solutions. Carcasses sprayed with 100 and 200 PPM chlorine showed significantly reduced *Salmonella* counts as compared with unsprayed controls. Carcasses washed with distilled water spray showed a significant reduction in *Salmonella typhimurium* counts. Within any given treatment, no significant difference was found between 5 and 30 minutes holding time.

The use of chlorine in poultry processing plants has been suggested by Ranken et al. (32) and Dawson et al. (10). The authors are agreed that bacterial counts are lowest when birds are cooled in ice water containing chlorine. A low concentration does not affect the acceptability of carcasses by the consumers.

Wabeck et al. (38) reported that a 20 and 40 PPM chlorine solution will destroy 10^3 *Salmonella* cells per ml within 30 minutes when organic matter such as poultry meat is absent. *Salmonella* artificially inoculated at 10^2 cells per ml on chicken drumsticks are slightly reduced by 20 and 40 PPM chlorine solution. Dixon and Pooley (11) reported that treatment of broiler chicken carcasses with 200 PPM of chlorine for 10 minutes usually prevented the subsequent recovery of *Salmonella* when fewer than 1000 organisms had been inoculated. When larger numbers of organisms were inoculated or when lower concentrations of chlorine were used, *Salmonella* were usually recovered from the treated carcasses.

Twenty PPM chlorine reduces the Salmonella on poultry carcasses has been reported by Nilsson and Regner (31). On the basis of taste panel evaluations they found the treatment does not influence the acceptability of the product. Taste panel evaluation of chicken fried after overnight storage was as high for the chlorine-treated product as for the untreated product.

Woodburn et al. (43) studied the efficacy of pasteurization before freezing precooked boned chicken. The chickens were packaged alone, with broth or with white sauce and were inoculated with approximately one million cells of Salmonella senftenberg, S. typhimurium, or one of the two food poisoning strains of Staphylococcus aureus, per g of food. Cooking in an electronic range or immersion in boiling water, as the source of heat, resulted in essentially a sterile product. The time necessary varied with the product and heat source. Taste panel comparisons of treated and untreated product indicated little difference in overall acceptability.

LYSOZYME

The most intensively studied enzyme has been lysozyme. It is a carbohydrase widely distributed in both the animal and plant kingdoms. Sensitivity to lysozyme varies with the bacterial species, Gram-positive organism having greatest sensitivity. In case of Gram-negative bacteria, the masking lipoprotein layer must be removed to

expose the lysozyme sensitive peptide glycon layer. A variety of means have been used to remove or damage the lipoprotein layer by the use of such chemical agents as trypsin, ethylenediaminetetraacetic acid (EDTA), sodium alkyle sulfate, etc. Of these EDTA has been most extensively used.

Wolin (42) reported that cell suspensions of Vibrio succinogenes are lysed by EDTA or lysozyme at alkaline pH. EDTA lysis leads to the formation of a cell ghost and lysozyme lysis leads to the formation of an empty round body.

Pseudomonas aeruginosa 64 has been extensively lysed by EDTA alone and destruction of cells is as complete as when lysozyme is used in combination with EDTA (12). Voss (37) reported that cells of Gram-negative bacteria undergo lysis when treated with lysozyme in the presence of EDTA and tris buffer. Gray and Wilkinson (17) showed that EDTA has a direct bacterial action against strains of Pseudomonas aeruginosa and Alcaligenes faecalis. The action of EDTA takes place at the cell wall of the organism.

IRRADIATION

The destruction of Salmonella and control of this widespread food pathogen group in certain foods by means of irradiation at low doses appears promising. Salmonella have been found to be the most radiation sensitive of all pathogenic organisms in foods. In general, an

adequate radiation dose for complete inactivation of Salmonella in most food is between 0.5 and 0.7 Mrad. Lower doses (e.g. 0.2 Mrad) are extremely effective in lowering numbers of Salmonella organisms in frozen meats, poultry, and eggs with negligible effects on organoleptic quality (21).

Although different meats vary considerably in their response to irradiation, the changes in color, odor, and flavor produced by sterilizing doses (4.8 Mrads) are usually sufficiently pronounced to render the process of doubtful commercial value. Pasteurizing doses of radiation up to 1 Mrad appreciably extend the shelf life of meat under chilled storage in many cases, producing little or no off flavors (7).

Heiligman et al. (19) produced a "First generation" irradiation sterilized chicken with 4.5-5.6 Mrad of gamma rays. Most important of the recognized deficiencies in the "First generation" product were "off" color, texture deterioration, and irradiation flavor.

Storage temperature and temperature at which irradiation is performed have a profound effect on the "off" color of the irradiated product. Hanson et al. (18) reported a taste panel readily detected the odor and flavor induced in chicken by 0.1 Mrad of irradiation at ambient temperature. Practically no irradiation odor and flavor formed when samples were irradiated at -20°C or lower in their experiment. Gernan et al. (16) reported that preference of cooked irradiated meat stored at room temperature became evident. They

concluded that cooking of meat prior to irradiation increased the storage ability.

Storage life of irradiated meat has been studied by some investigators. Heiligman (20) showed that irradiated foods remained stable in storage. Chicken parts, fresh roasts, pork, bacon, barbecued pork and pork chops scored approximately the same after 18-24 months at 70°F as they did initially (shortly after irradiation). At 100°F, some of these items began to decrease in acceptability after 9-12 months. Licciardello et al. (24) reported that the appearance of various meat samples after 12 years of storage was excellent. Some white specks were present on the surface of beef steak and hamburger. A slight irradiation odor was perceptible but it was not considered objectionable.

CHAPTER III

EXPERIMENTAL PROCEDURES

I. Chemical pasteurization of turkey skin samples using Salmonella senftenberg 775W as the test organism

A frozen stock culture of Salmonella senftenberg 775W was thawed, mixed, and incubated at 37°C for 24 hours. A 3 mm loopful of the culture was then transferred to 100 ml sterile trypticase soy broth + 2% yeast extract and incubated at 37°C for 12 hours. The culture was diluted in deionized water. The contaminated fluid (8×10^3 to 8×10^5 viable cells per ml) was prepared in 100 ml aliquots just prior to contaminating the skin samples.

Solutions of different pasteurizing chemicals (lactic acid and sodium carbonate) were prepared in distilled water in volumetric flasks. The pH of the solution was measured with a pH/mv Electrometer model 245. A separate 250 ml aliquot of pasteurizing solution was used for each skin sample. The solutions, placed in beakers, were heated to the desired temperature on hot plates.

Skin samples (7.145 cm^2) were taken from turkey breasts, drumsticks and wings with a sterile coring pipe. The skin was removed with sterile scissors and forceps. The skin samples were immersed in the contaminating fluid and swirled for two minutes at

room temperature. The samples were removed with sterile forceps, placed in sterile plastic plates, and held for 30 minutes to allow the adjustment of the organism on the skin before pasteurization.

The contaminated skin samples were immersed in 250 ml of the pasteurizing solution. Different temperature, time, and concentration of solutions were tested during pasteurization. The skin samples were removed from the pasteurizing solution aseptically and placed in sterile blender jars (capacity 500 ml). As the samples were removed from the pasteurizing fluids, they were examined, and any change in physical appearance, such as color, was recorded.

The control, one skin sample per experiment was inoculated with the test organism but not pasteurized. Uninoculated skin samples were also analyzed for Salmonella to assure that samples were Salmonella-free. Controls were held at $27^{\circ} \pm 2^{\circ} \text{C}$.

Two hundred and fifty ml of sterile lactose broth was poured on each sample aseptically and blended for two minutes. The blended solution was then transferred to sterile 500 ml jars. Tergitol^{*} anionic 7 (1.5 ml) was added to the blended solution which was then incubated at $37 \pm 1^{\circ} \text{C}$ for 24 hours.

The 24 hour incubated lactose culture was agitated gently to ensure homogeneity. Ten ml of this lactose culture was removed aseptically and added to 100 ml of Selenite cystine

* A detergent from Sigma Chemical Co., St. Louis.

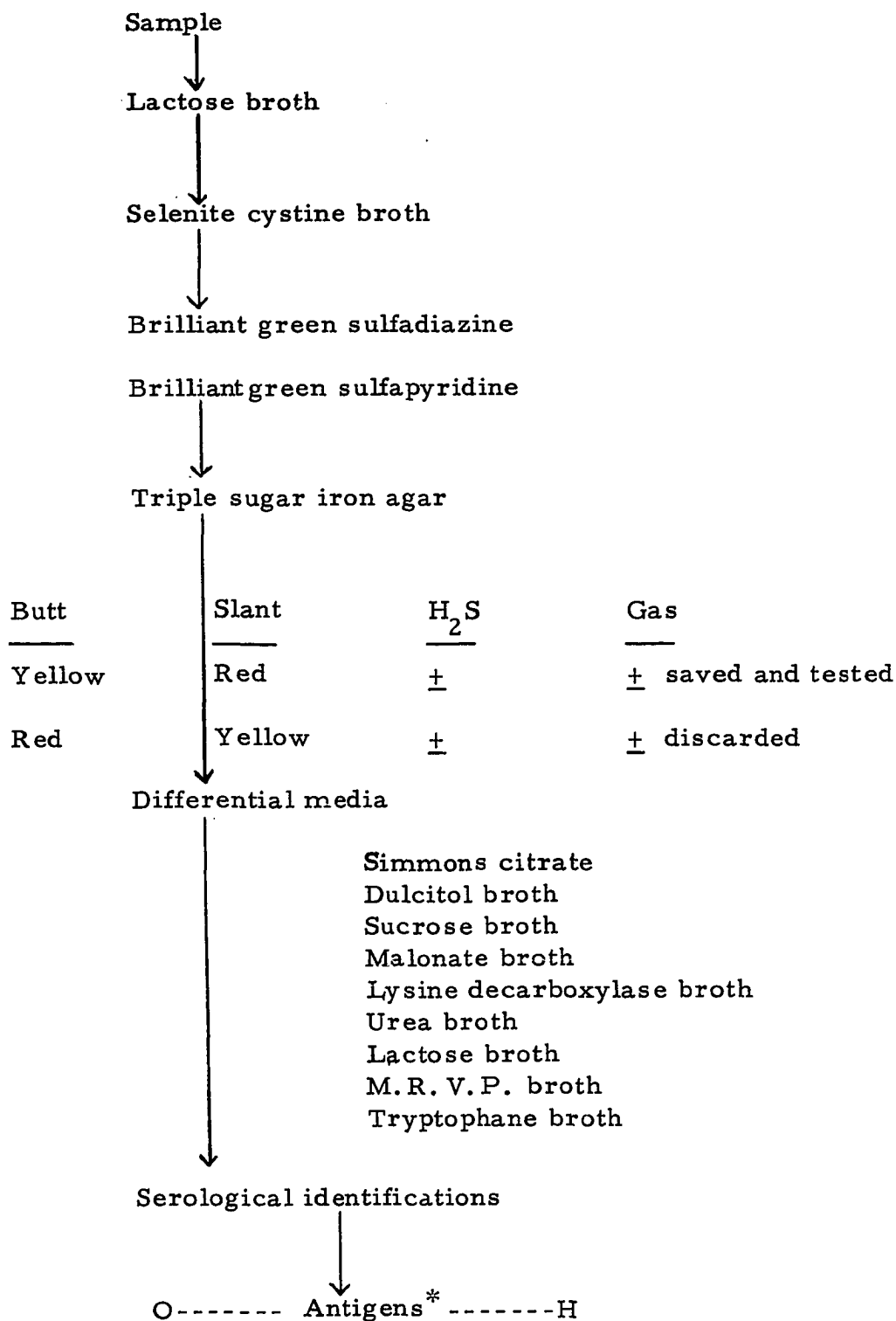
enrichment broth which was then incubated for 24 hours at $37 \pm 1^{\circ}\text{C}$ on a gyratory shaker-incubator at 150 rpm. BGSP (Brilliant Green Sulfapyridine) and BGSD (Brilliant Green Sulfadiazine) agar plates were streaked from the enrichment culture. Standard AOAC (3) methods were followed for final identification of the organism. The test organism, S. senftenberg 775W was deemed present if positively identified according to the following criterion: H_2S negative, group E_4 (modified Kaufman White scheme) positive and had typical biochemical reactions of Salmonella. A schematic diagram of the entire procedure is shown in Figure 1.

II. Chemical pasteurization of turkey drumsticks contaminated with Salmonella senftenberg 775W

The culture of Salmonella senftenberg 775W was prepared as described in Part I. The culture in the stationary growth phase was diluted in 0.1% sterile peptone water. The contaminating fluid (5×10^5 to 8×10^5 viable cells per ml) was prepared in 500 ml aliquots, just prior to contaminating the drumstick samples. The number of viable cells of the culture was determined by aerobic plate counts.

Pasteurizing solutions of different chemicals (lactic acid, acetic acid, sodium carbonate, potassium hydroxide, and sodium chloride) were prepared with distilled water in volumetric flasks. Three thousand to 4000 ml of pasteurizing fluid were required to completely immerse a drumstick, depending on the size of the drumsticks. The

Figure I. Schematic diagram for identification of Salmonella.



*Polyvalent, E₄ and B antisera were used for serological typing.

solution was steamed in stainless steel containers with aluminum foil cover in the autoclave.

Medium size (approximately 500 g) turkey drumsticks were obtained from Longmont Turkey Processing plant. The drumsticks were thawed for 24 hours at room temperature in double paper grocery bags to avoid introduction of extraneous organisms and provide homogeneous thawing. The drumsticks were washed in cold tap water. A 500 ml aliquot of contaminating fluid was poured in the plastic container (capacity 900 ml). Each drumstick was placed in the plastic container containing contaminating fluid and swirled for two minutes at room temperature. Plastic, sterile disposable gloves were worn while swirling the drumsticks. The drumsticks were removed from the contaminating fluid and held for 60 minutes in sterile cryovac bags to allow the adjustment of the organism on the drumsticks before pasteurization.

The contaminated drumsticks were immersed in pasteurizing solution. Different combinations of temperature, time and concentration of solution were tested during the pasteurization. The drumsticks were removed from the pasteurizing solutions aseptically and placed in sterile jars (capacity 4000 ml). As the drumsticks were removed from the pasteurizing fluids, they were examined, and any change in physical appearance, such as color, was recorded. The control, one drumstick per experiment, was treated exactly as the experimental drumsticks, except that immersion was in distilled water.

One liter of sterile lactose broth was poured on each drumstick aseptically in the jars. Tergitol anionic 7 (6 ml) was added to the lactose broth which was then incubated at $37 \pm 1^{\circ}\text{C}$ for 24 hours.

The 24 hour incubated lactose culture was agitated gently to ensure homogeneity. Ten ml of this lactose culture was removed aseptically and added to 100 ml of sterile selenite cystine enrichment broth which was then incubated for 24 hours at $37 \pm 1^{\circ}\text{C}$ on a gyratory shaker-incubator at 150 rpm or in an ordinary incubator at the same temperature. BGSP and BGSD agar plates were streaked from the enrichment culture. The test organism, S. senftenberg 775W was identified as described in Part I.

III. X-irradiation of whole turkey carcasses and turkey drumsticks contaminated with Salmonella senftenberg 775W

The culture of Salmonella senftenberg 775W was prepared as described in Part I. The culture was diluted as described in Part II. The contaminating fluid (5×10^5 to 8×10^5 viable cells per ml) was prepared in 500 ml aliquots, just prior to contaminating the whole turkey carcasses or turkey drumsticks. Viable cell counts of the culture was determined by aerobic plate count.

Frozen small, young, white turkey hen carcasses (approximately 3500 g) and medium size drumsticks (approximately 500 g) were obtained from Longmont Turkey Processing plant. The carcasses or drumsticks were thawed in their plastic packages in double paper

grocery bags at room temperature for 24 hours. The procedure avoided the introduction of extraneous organisms and gave homogenous thawing. The carcasses or drumsticks were rinsed in cool tap water, giblets removed and placed in sterile plastic cryovac bags.

A 500 ml aliquot of contaminating fluid was poured in the plastic bag containing whole carcass or drumstick. The plastic bag containing the carcass or drumstick and contaminating fluid was shaken for two minutes, assuring complete exposure of the carcass or drumstick to the test organism. The contaminating fluid was poured off and carcass or drumstick was held in the closed bag at room temperature for 60 minutes to allow the adjustment of the organism on the carcass or drumstick before pasteurization. The carcass or drumstick was aseptically removed from the plastic bags and packed similarly to commercial packaging except that air was not evacuated from the bag. The packaged carcass or drumstick was placed in a hard plastic container to be used during X-irradiation.

The irradiation was performed using a General Electric Maxitron 300 X-ray machine, operated at 300 KVP, 20MA with a half value layer of 2.8 mm. Cu delivering 268 rads per minute at an effective energy of 49 KEV. Each carcass or drumstick was placed in the center area of the X-ray beam to assure complete irradiation of the carcass or drumstick. Each carcass or drumstick was irradiated for a specified period of time. The temperature in the X-ray room varied between 20 to 24°C.

Each irradiated carcass or drumstick was aseptically transferred from its packaging to a sterile stainless steel container. As the drumstick or carcass was removed from its packaging, it was examined, and any observable change in physical appearance, such as color, was recorded. The control, one carcass or drumstick, was inoculated with the test organism but not treated.

A 2000 ml amount of sterile selenite cystine broth was poured onto the whole carcass in the container and was covered with aluminum foil. The drumsticks were transferred to sterile jars (capacity 4000 ml) containing 1000 ml of sterile selenite cystine broth. The containers and jars containing carcass and drumstick with enrichment were incubated at $37 \pm 1^{\circ}\text{C}$ for 24 hours. BGSP and BGSD agar plates were streaked from the enrichment culture. The test organism, S. senftenberg 775W was identified as described in Part I.

IV. Microwave irradiation of broiler carcasses and turkey drumsticks, contaminated with Salmonella senftenberg 775W

The culture of Salmonella senftenberg 775W was prepared as described in Part I. The culture was diluted as described in Part II. The contaminating fluid (5×10^5 to 8×10^5 viable cells per ml) was prepared in 500 ml aliquots, just prior to contaminating the broiler carcass or turkey drumstick. The number of viable cells of the organism was determined by aerobic plate counts.

Unfrozen, broiler carcasses (approximately one Kg) were obtained from a local grocery store. The turkey drumsticks were obtained from Longmont Turkey Processing plant and thawed in a double paper grocery bag for 24 hours at room temperature to avoid any extraneous contamination. Broiler carcasses, with giblets removed, and turkey drumsticks were washed in cold tap water and placed in plastic cryovac bags. Carcasses or drumsticks were contaminated as described in Part III. Each carcass or drumstick was aseptically transferred into a glass pan and covered with a sterile Saran wrap.

Microwave irradiation was performed using a Minute Master Electronic Oven, Model 350.004. The carcasses or drumsticks were irradiated in the electronic oven for a specific period of time. Each carcass or drumstick was aseptically removed from the glass pan and transferred aseptically to a sterile stainless steel container or sterile jars. As the carcass or drumstick was removed from the glass pan, they were examined, and any change in physical appearance, such as color, was recorded. The control, one carcass or drumstick, was inoculated with the test organism but not treated.

A 1500 ml aliquot of sterile selenite cystine broth was poured onto the broiler carcass in the sterile container then the container was covered with aluminum foil. The drumsticks were transferred to sterile jars (capacity 4000 ml) containing 1000 ml of sterile selenite

cystine broth. Any outside contamination was avoided. The containers and jars containing a carcass or drumstick with enrichment broth were incubated at $37 \pm 1^{\circ}\text{C}$ for 24 hours. BGSP and BGSD agar plates were streaked from the enrichment culture. The test organism, S. senftenberg 775W was identified as described in Part I.

V. Enzymatic and chemical treatment of turkey drumsticks contaminated with Salmonella senftenberg 775W or S. typhimurium

The culture of S. senftenberg 775W was prepared and diluted as described in Parts I and II. The culture of S. typhimurium was prepared and diluted similar to S. senftenberg 775W. The contaminated fluid (8×10^5 to 8×10^5 viable cells per ml) was prepared in 500 ml aliquots, just prior to contaminating the drumsticks. The number of viable cells of the organism was determined by aerobic plate counts.

Solutions of lysozyme, ethylenediaminetetraacetic acid (EDTA), chlorine and sodium borate were prepared with distilled water in volumetric flasks. A problem of solubility was noticed only in sodium borate solution. The desired quantity of sodium borate was dissolved in 500 ml hot distilled water on a hot plate. The 500 ml aliquot of dissolved sodium borate solution was added to enough distilled water to achieve the desired sodium borate concentration. Three thousand to 4000 ml of pasteurizing solution was required to completely immerse a drumstick, depending on the size of drumsticks.

Medium size (approximately 500 g) turkey drumsticks were obtained from Longmont Turkey Processing plant. Drumsticks were thawed and washed as described in Part II. A 500 ml aliquot of contaminating fluid of S. senftenberg 775W or S. typhimurium was poured into plastic bags containing the drumsticks. The plastic bags containing drumsticks and contaminating fluid were shaken for two minutes, assuring complete exposure of the drumstick to the test organism. The contaminating fluid was poured off and drumsticks were held in the closed bag at room temperature for 60 minutes to allow the adjustment of the organism to the drumstick environment before treatment.

The contaminated drumsticks were immersed in the pasteurizing solution. Different temperatures, times, and concentrations of solutions were tested during the treatment. The drumsticks were removed from the pasteurizing solutions aseptically and placed in sterile jars (capacity 4000 ml). As the drumsticks were removed from the pasteurizing fluids, they were examined, and any change in physical appearance, such as color, was recorded. The control, one drumstick per experiment, was inoculated with the test organism but not pasteurized and was immersed in plain distilled water under the same time and temperature conditions as treated drumsticks.

A 1000 ml quantity of sterile selenite cystine broth was poured onto the drumsticks in a sterile jar, incubated at $37 \pm 1^{\circ}\text{C}$ for 24 hours. BGSP and BGSD agar plates were streaked from the enrichment culture.

The test organism S. senftenberg 775W was identified as described in Part I.

The test organism S. typhimurium was deemed present if positively identified according to the following criterion; H₂S positive, group B (modified Kaufman White scheme) positive and had typical biochemical reactions of Salmonella.

Taste Panel Evaluation

Uninoculated turkey drumsticks were immersed in solutions of 3% sodium borate, 6% sodium chloride, 4% sodium carbonate, and water for three hours at room temperature. The drumsticks were then roasted at 350^oF for 45 minutes. The cooked meat was cut into small pieces and served to an informal taste panel. Panel members recorded their opinion about taste, flavor, and tenderness of the meat.

CHAPTER IV

RESULTS AND DISCUSSION

Chemical pasteurization of turkey skin samples

The first chemical tested on skin samples was lactic acid. Solutions of lactic acid, ranging from 0.5 to 4.5 percent, destroyed Salmonella senftenberg 775W and normal flora of skin in 15 sec at 88°C, as evidenced by no growth on BGSD and BGSP plating media (Table 1). Two to 4.5 percent solutions of lactic acid resulted in the skin appearing cooked, whereas lower concentrations of lactic acid (0.5 to 1.5%) produced partially cooked skin. The temperature of lactic acid solution was probably too high (88°C) making it difficult to specifically attribute the destruction of the organism to lactic acid, time, heat of the solutions, or combinations of agents.

Immersing the skin samples in lactic acid solutions (0.5 to 2.5%) eliminated S. senftenberg 775W and other aerobic bacteria in 15 sec at 82°C, as evidenced by no growth on BGSP and BGSD plating media (Table 1a). This treatment resulted in partially cooked appearance of the skin. A 0.5 percent solution of lactic acid reduced S. senftenberg 775W to an undetectable level irrespective of temperature (66 to 71°C) and time (15 and 30 sec). Temperatures of 66 and 71°C resulted in partial cooking of the skin samples when samples were immersed in

TABLE 1. PASTEURIZATION OF TURKEY SKIN SAMPLES CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING LACTIC ACID SOLUTIONS (88°C, 15 SEC).

Sample No.	Lactic acid as percent of the solution	Salmonella isolated ^a	Skin score after pasteurization ^b
1	4.5	- ¹	3
2	4.0	- ¹	3
3	3.5	- ¹	3
4	3.0	- ¹	3
5	2.5	- ¹	3
6	2.0	- ¹	2
7	1.5	- ¹	2
8	1.0	- ¹	2
9	0.5	- ¹	2
Control	inoculated no pasteurization	+	1
Control	no inoculation no pasteurization	-	1
Control	no inoculation no pasteurization	-	1

^aThe test organism, S. senftenberg 775W was deemed present if positively identified according to the following criterion; H₂S negative, group E₄ positive (modified Kaufman White scheme) and typical biochemical reactions of Salmonella.

+ Salmonella isolated

- Salmonella not isolated

- ^b
1. Normal appearance
 2. Partially cooked appearance
 3. Cooked appearance

¹No bacterial growth on BGSP or BGSD plating media.

TABLE 1a. PASTEURIZATION OF TURKEY SKIN SAMPLES CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING LACTIC ACID SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Lactic acid as percent of the solution	Temperature (°C)	Time (sec.)		
1	2.5	82	15	- ¹	2
2	2.0	82	15	- ¹	2
3	1.5	82	15	- ¹	2
4	1.0	82	15	- ¹	2
5	0.5	82	15	- ¹	2
6	0.5	71	15	-	1
7	0.5	71	30	-	2
8	0.5	66	15	-	1
9	0.5	66	30	-	2
10	0.5	65	30	-	2
11	0.5	60	30	+	2
12	0.5	60	30	+	2
13	0.5	60	45	-	3
Control	inoculated no pasteurization			+	1
Control	inoculated no pasteurization			-	1
Control	no inoculation no pasteurization			-	1
Control	no inoculation no pasteurization			-	1
Control	no inoculation no pasteurization			-	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cRefer to footnote "1" of Table 1.

lactic acid solution for more than 15 sec. Even though the test organism was not isolated from the 66 or 71°C for 15 sec treatment, the treatment did not produce sterile skin, as evidenced by green colonies on BGSP and BGSD plating media. AOAC (3) suggested picking green colonies to isolate *Salmonella* when typical pink colonies are absent on BGSP or BGSD. When green colonies were inoculated into TSI (Triple Sugar Iron agar) and incubated for 24 hours at 37°C, only atypical reactions for *Salmonella* were noted. In most cases a large amount of gas was produced, causing the TSI media to push towards the cap and even to be forced out of the tube. This created contamination problems and therefore further use of green colonies for identification of *Salmonella* was discontinued.

A 0.5 percent lactic acid solution failed to eliminate *S. senftenberg* 775W in 30 sec at 60°C (Table 1a). This concentration proved effective in eliminating the test organism when immersion time of the skin was increased to 45 sec at 61°C, but this immersion produced partially cooked skin samples. The effectiveness of the lactic acid treatment was increased when the temperature of the solution was increased (65°C) and skin was immersed at this temperature for 30 sec.

The pH of the 0.1 to 0.5 percent lactic acid solutions was approximately 2.1 to 2.7, while pH of the 1 to 4 percent solution was 1.4 to 1.9 (Table 4). Lactic acid, having solvent properties, helped

remove dirt and fat particles present on the skin, thus exposing the organism to treatment. The bacteriocidal activity of lactic acid was elucidated by Miller et al. (28). They reported that 3 percent lactic acid at 26°C would effectively eliminate the *Salmonella* culture of 10^5 after 5 minutes exposure time. Avens (4) pointed out that lactic acid (5%) was most effective in lowering *S. senftenberg* 775W to less than detectable levels and reducing the total bacteria on skin samples.

Solutions of sodium carbonate, ranging from 0.5 to 3 percent, destroyed *S. senftenberg* 775W and normal flora of the skin in 15 sec at 88°C, as evidenced by no growth on BGSP and BGSD plating media (Table 2). At 79°C, 1.5 and 2 percent solutions of sodium carbonate destroyed the test organism in 15 sec without affecting the skin quality. A concentration of 0.5 to one percent sodium carbonate solution failed to reduce the test organism to an undetectable level under the same temperature and time.

At 77°C, one percent solution of sodium carbonate eliminated the test organism on skin samples in 15 sec without affecting the skin quality, but under the same conditions for 30 sec, the skin was partially cooked (Table 2a). When one percent solutions of sodium carbonate at temperatures between 60 and 71°C, for the various length of time were tested, either the organism was killed and the skin was cooked, or the skin was normal and organism was not killed. When the concentration of the sodium carbonate solution was increased to two percent, it

killed the organism at 65 and 71^oC in 15 sec without affecting the appearance of the skin.

The bacteriocidal activity of sodium carbonate was studied by Miller et al. (28). They pointed out that sodium carbonate was the most effective of the alkaline products tested for elimination of *Salmonella*, however they did not use sodium carbonate in the presence of organic matter such as poultry skin or meat. The pH of sodium carbonate solutions used in this study ranged between 9.5 to 11.4 (Table 4).

Pasteurization of skin samples was next attempted using hot water (Table 3). Limited success was achieved by immersing skin samples in water at 60 and 66^oC. Water at 60 and 66^oC failed to eliminate the test organism in 15 sec but was effective in 30 sec. Complete elimination of *S. senftenberg* 775W was achieved by immersing skin samples in hot water between 77 to 88^oC for 15 sec. These observations are in agreement with Avens (4) who reported that exposure of skin to tap water at 87.8^oC for 15 sec reduced *S. senftenberg* 775W to less than detectable levels. Water failed to eliminate *S. senftenberg* 775W in 15 sec at 71^oC but destroyed it in 30 sec at the same temperature. A water temperature of 82-88^oC in 15 sec caused partial cooking of the skin.

In some trials *S. senftenberg* 775W could not be isolated from the control groups. This may have been due to the overgrowth of

TABLE 2. PASTEURIZATION OF TURKEY SKIN SAMPLES CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM CARBONATE SOLUTIONS (15 SEC).

Sample No.	Pasteurization		Salmonella isolated ^a	Skin score ^b
	Sodium carbonate as percent of the solution	Temperature (°C)		
1	3.0	88	- ¹	2
2	2.5	88	- ¹	2
3	2.0	88	- ¹	2
4	1.5	88	- ¹	2
5	1.0	88	- ¹	2
6	0.5	88	- ¹	2
7	2.0	79	- ¹	1
8	1.5	79	-	1
9	1.0	79	+	1
10	0.5	79	+	1
Control	no inoculation no pasteurization		-	1
Control	inoculated no pasteurization		+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cRefer to footnote "1" of Table 1.

TABLE 2a. PASTEURIZATION OF TURKEY SKIN SAMPLES CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM CARBONATE SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Sodium carbonate as percent of the solution	Temperature (°C)	Time (sec.)		
1	1	77	15	-	1
2	1	77	30	-	2
3	1	71	15	+	1
4	1	71	30	-	2
5	1	71	45	-	3
6	1	65	30	-	2
7	1	65	45	-	3
8	1	60	30	-	2
9	1	60	45	-	3
10	2	71	15	-	1
11	2	65	15	-	1
Control	no inoculation no pasteurization			-	1
Control	no inoculation no pasteurization			-	1
Control	inoculated no pasteurization			-	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

TABLE 3. PASTEURIZATION OF TURKEY SKIN SAMPLES CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING HOT WATER.

Sample No.	Pasteurization		Salmonella isolated ^a	Skin score ^b
	Temperature (°C)	Time (sec.)		
1	88	15	-	2
2	82	15	-	2
3	77	15	-	1
4	71	15	+	1
5	71	30	-	2
6	71	30	-	2
7	66	15	+	1
8	66	30	-	2
9	60	15	+	1
10	60	30	-	2
11	60	45	-	3
Control	no inoculation no pasteurization		-	1
Control	no inoculation no pasteurization		-	1
Control	inoculated no pasteurization		-	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

TABLE 4. pH OF SODIUM CARBONATE AND LACTIC ACID SOLUTIONS USED AS PASTEURIZING FLUID.

Sample No.	Chemical	Percent of solution	pH
1	Lactic acid	0.1 to 0.5	2.1 to 2.7
2	Lactic acid	1.0 to 4.0	1.4 to 1.9
3	Sodium carbonate	0.001 to 0.01	9.5 to 10.5
4	Sodium carbonate	0.1 to 0.5	11.0 to 11.3
5	Sodium carbonate	1.0 to 2.0	11.4

normal flora of the skin which inhibited the growth of the test organism. Such phenomena is in agreement with Avens (4) who also had problems in isolating Salmonella from control skin samples. No Salmonella was isolated from uninoculated, nonpasteurized skin samples, indicating the skin was not already contaminated with Salmonella serotypes.

Lactic acid pasteurization of turkey drumsticks

Immersing the drumsticks in lactic acid solution (0.25 to one percent) at 82° C for 15 sec, failed to eliminate S. senftenberg 775W from turkey drumsticks (Table 5). This combination of temperature, time, and solution proved very effective in destroying the test organism on skin samples (Table 1a), suggesting that some of the organisms remained protected in the larger number of cuts and feather follicles of the drumsticks and never came in direct contact with the pasteurizing solution.

A 0.5 percent solution of lactic acid eliminated the test organism on turkey drumsticks in 45 sec at 73° C. These observations are contrary to Avens (4), who isolated Salmonella, group E₄, H₂S negative from four of four artificially contaminated turkey drumsticks after pasteurization in 5% lactic acid at 71.7° C regardless of exposure times ranging from 5 to 300 sec. The difference could have been due to differences in temperatures of pasteurizing solutions, properties of the drumsticks, or swirling the drumsticks in pasteurizing solutions.

TABLE 5. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING LACTIC ACID SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Lactic acid as percent of the solution	Temperature (°C)	Time (sec.)		
1	0.25	82	15	+	2
2	0.50	82	15	+	2
3	0.75	82	15	+	2
4	1.00	82	15	+	2
5	control ^c	82	15	+	2
6	0.5	73	45	-	2
7	0.5	62	60	-	1
8	0.5	62	180	-	2
9	1.0	62	180	-	2
10	control ^c	62	100	-	2

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cThe control consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as lactic acid pasteurized drumsticks.

The drumsticks were swirled in the pasteurizing fluids in the experiments reported herein. It may be that swirling of the drumsticks brought the test organism in direct contact with the pasteurizing solution, thus killing or reducing the test organism to undetectable levels.

A 0.5 percent solution of lactic acid eliminated the test organism in 60 sec at 62°C without affecting the carcass quality. When exposure time was increased to 180 sec at 62°C the drumstick quality was impaired and a partially cooked carcass was observed.

The sensitivity of S. senftenberg 775W to lactic acid solution (0.5 to 2.0%) at higher temperature (88°C) is shown in Table 5a. The test organism could not be isolated when drumsticks were immersed for 15 sec irrespective of concentration of lactic acid solution (0.5 to 2%). An immersion time from 15 to 210 sec at 88°C produced partially cooked appearance of the drumsticks irrespective of concentration of solution (0.5 to 2%). The test organism was not isolated from the control drumstick immersed in water for 15 sec at 88°C.

At this point it appeared that it was the higher temperature of the solution which affected the skin and meat of the drumsticks and not the concentration of lactic acid in pasteurizing fluid. It was therefore decided to conduct treatment at a lower temperature.

Three to four percent solution of lactic acid at 32°C for 90 minutes eliminated S. senftenberg 775W on turkey drumsticks, whereas a two percent solution of lactic acid failed to eliminate the test organism under the same conditions (Table 5a). In all treatments the

TABLE 5a. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING LACTIC ACID SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Lactic acid as percent of the solution	Temperature (°C)	Time		
1	1	88	15 sec.	-	3
2	1	88	15 sec.	-	3
3	0.5	88	15 sec.	-	3
4	1	88	15 sec.	-	3
5	2	88	15 sec.	-	3
6	control ^c	88	15 sec.	-	2
7	1	32	90 min.	+	3
8	2	32	90 min.	+	3
9	3	32	90 min.	-	3
10	4	32	90 min.	-	3
11	control ^c	32	90 min.	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cRefer to footnote "c" of Table 5.

drumsticks appeared like a pickled cucumber. They were very soft and off color. A typical lactic acid odor was detected in the treated drumsticks.

Lactic acid is commonly used in the manufacture of lactates which are widely used in food products and in medicine. It is an edible organic acid and is quite effective in eliminating *Salmonella* and other bacteria from turkey drumsticks, but it changes the physical appearance of the meat and skin in a way which may not be accepted by the consumers.

Acetic acid pasteurization of turkey drumsticks

Acetic acid proved to be less effective in eliminating *S. senftenberg* 775W than lactic acid (Table 6). The superiority of lactic acid over acetic acid in reducing bacterial load on chicken carcasses was reported by Miller *et al.* (28). One percent solutions of acetic acid and water eliminated *S. senftenberg* 775W from turkey drumsticks at 88°C in 15 sec but produced a slightly cooked appearance of the drumsticks. A four percent solution of acetic acid eliminated the test organism at 32°C in 90 minutes on turkey drumsticks whereas a three percent solution failed to eliminate the test organism under the same conditions.

Higher concentration of acetic acid, lower temperature of solution, and longer immersion time produced "off color" drumsticks. Drumsticks resembled pickled cucumbers, as noticed with lactic acid.

TABLE 6. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING ACETIC ACID SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Acetic acid as percent of the solution	Temperature (°C)	Times		
1	2	88	210 sec.	-	3
2	1	88	60 sec.	-	3
3	1	88	15 sec.	-	2
4	control ^c	88	15 sec.	-	2
5	1	32	90 min.	+	3
6	3	32	90 min.	+	3
7	4	32	90 min.	-	3
8	control ^c	32	90 min.	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cThe control consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as acetic acid pasteurized drumsticks.

A typical acetic acid odor was noticed in the room where the experiment was performed. This odor was also noticed in treated drumsticks. Acetic acid is an excellent solvent for many compounds, is commonly used in preserving foods and widely used in commercial organic synthesis. It is an edible organic acid and can be used for pasteurizing poultry meat.

Potassium hydroxide pasteurization of turkey drumsticks

The sensitivity of S. senftenberg 775W to potassium hydroxide on turkey drumsticks is presented in Table 7. The use of this chemical proved unfruitful in pasteurizing turkey drumsticks. Potassium hydroxide has a potential caustic effect on tissues and the test organism. It was hypothesized that a low concentration of potassium hydroxide would destroy the test organism on turkey drumsticks. Acetic acid would be used to neutralize the caustic effect of potassium hydroxide.

At 88°C, water and all solutions of potassium hydroxide reduced S. senftenberg 775W to an undetectable level on turkey drumsticks in 15 sec. At lower temperature (82°C), 0.5 percent solution of potassium hydroxide failed to eliminate the test organism in 60 sec. A higher concentration of potassium hydroxide (1 to 1.5%) eliminated the test organism from drumsticks under the same temperature and time. Two percent solution of potassium hydroxide failed to eliminate S. senftenberg 775W at 74°C in 30 sec.

TABLE 7. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING POTASSIUM HYDROXIDE SOLUTIONS.

Sample No.	Pasteurization			Salmonella isolated ^a	Skin score ^b
	Potassium hydroxide as percent of the solution	Temperature (°C)	Time (sec.)		
1	2.0	88	210	-	3
2	1.0	88	60	-	3
3	1.0	88	30	-	3
4	1.0	88	15	-	2
5	control ^c	88	15	-	2
6	2.0	82	60	-	3
7	1.5	82	60	-	3
8	1.0	82	60	-	3
9	0.5	82	60	-	3
10	control ^c	82	60	+	3
11	2.0	74	30	+	2
12	1.5	74	30	+	2
13	1.0	74	30	+	2
14	0.5	74	30	+	2
15	control ^c	74	30	+	2

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cThe control consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as potassium hydroxide pasteurized drumsticks.

A high temperature or a high concentration of potassium hydroxide changed the physical appearance of the drumsticks. The treated drumsticks appeared partially cooked and red in color. Redness of the drumsticks could be a chemical reaction with the potassium hydroxide. Noting the detrimental effects the potassium hydroxide had on drumsticks, the acetic acid neutralization was not tested.

Lysozyme and EDTA pasteurization of turkey drumsticks

The effect of lysozyme and EDTA as pasteurizing agents for turkey drumsticks contaminated with S. senftenberg 775W is reported in Table 8. At 74°C, 0.5 percent lysozyme or 0.05 percent lysozyme plus 0.5% EDTA or 0.5% EDTA eliminated the test organism in 45 sec. Lysozyme and EDTA were proven quite effective in eliminating the test organism at 63°C.

A slight change in selenite cystine broth color was observed when pasteurized drumsticks treated with lysozyme were incubated at 37°C for 24 hours. Deep red color of selenite cystine broth was observed in control drumsticks. This suggested that lysozyme reduced S. senftenberg 775W on drumsticks. It probably also reduced the normal flora of drumsticks.

Lysozyme, being a protein, could be denatured at temperatures as high as 63°C. With this in mind it is difficult to attribute the elimination of Salmonella to the lysozyme, when it feasibly could have been the high temperature alone that caused the elimination. It was

TABLE 8. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING LYSOZYME AND EDTA SOLUTIONS.

Sample No.	Lysozyme and EDTA as percent of the solution	Temperature (°C)	Time	Salmonella isolated ^a	Skin score ^b
1	0.5% lysozyme	74	45 sec.	-	1-2
2	0.05% lysozyme + 0.5% EDTA	74	45 sec.	-	1-2
3	0.5% EDTA	74	45 sec.	-	1-2
4	control ^c	74	45 sec.	+	1-2
5	0.05% lysozyme	63	180 sec.	-	2
6	0.05% lysozyme + 0.5% EDTA	63	180 sec.	-	2
7	0.05% lysozyme	63	60 sec.	-	1
8	0.05% lysozyme + 0.5% EDTA	63	60 sec.	-	1
9	0.5% EDTA	63	60 sec.	-	1
10	control ^c	63	60 sec.	+	1
11	0.1% lysozyme	32	90 min.	+	1
12	0.1% lysozyme	21 ± 2	180 min.	-	1
13	0.5% EDTA	21 ± 2	180 min.	+	1
14	0.1% lysozyme + 0.5% EDTA	21 ± 2	180 min.	-	1
15	0.05% lysozyme + 1.0% EDTA	21 ± 2	180 min.	-	1
16	control ^c	21 ± 2	180 min.	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cControl consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as lysozyme and EDTA pasteurized drumsticks.

therefore decided to conduct experiments with lysozyme at lower temperature. A 0.1 percent solution of lysozyme failed to eliminate the test organism at 32°C in 90 minutes, whereas 0.1 percent lysozyme solution eliminated the test organism at 21°C ± 2 in 180 minutes. EDTA, alone did not eliminate the test organism but when used with lysozyme, the combination reduced the test organism to an undetectable level.

To test the efficacy of lysozyme, a S. senftenberg 775W culture in stationary growth phase (8×10^3 to 8×10^5 viable cells per ml of culture) was prepared. One hundred ml of 0.1% lysozyme solution was aseptically added to 100 ml of S. senftenberg 775W culture. As a control, 100 ml of sterile distilled water was added to another 100 ml of Salmonella culture. These solutions were incubated at 27°C. BGSP agar plates were streaked at 2, 3 and 6 hours intervals. No growth on BGSP was observed from the lysozyme treated culture, whereas the test organism was isolated from the control group.

This experiment proved that lysozyme is effective in destroying Salmonella. Lysozyme is a mucolytic enzyme with bacteriocidal properties that may be used safely in a pasteurizing solution for the destruction of Salmonella and other organisms.

X-irradiation of whole turkey carcasses and turkey drumsticks

The effect of X-irradiation on the destruction of S. senftenberg 775W on whole turkey carcasses and turkey drumsticks is presented

in Table 9. Eighty thousand rads of X-ray failed to eliminate the test organism on whole turkey carcasses but reduced the test organism to an undetected level on turkey drumsticks.

It appeared from these data that the total mass was the main factor in not eliminating the test organism from whole turkey carcasses. Turkey drumsticks and whole carcasses were contaminated with the same amount of inoculum and were irradiated under the same environmental conditions.

Coleby (7) reported that odor of the raw meat is one of the most sensitive sensory tests, and it is often possible to detect changes in odor after doses of irradiation as low as 50,000 rads. No odor was detected in this study. The difference could have been due to the sensitivity of sensory tests, age of the meat or temperature at which irradiation was performed. Chicken meat suffers relatively less organoleptic change on irradiation than beef or pork (7); therefore, it can be irradiated without much damage to organoleptic quality of the meat.

A 50 ml diluted culture of S. senftenberg 775W (5×10^5 to 8×10^5 viable cells per ml of culture) in stationary growth phase was irradiated with 80,000 rads of X-ray in sterile petriplates. A 50 ml culture containing the same number of cells was kept at room temperature as control. BGSP agar plates were streaked from irradiated and control culture. No growth from irradiated culture was observed, however the test organism was isolated from the control culture.

TABLE 9. X-IRRADIATION OF WHOLE TURKEY CARCASSES AND TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W.

Sample No.	Whole turkey carcass or turkey drumstick	Total X-ray (rads)	Salmonella isolated ^a
1	whole carcass	30,000 r	+
2	whole carcass	40,000 r	+
3	whole carcass	50,000 r	+
4	whole carcass	80,000 r	+
5	control (whole carcass) ^b	--	+
6	drumsticks	80,000 r	-
7	drumsticks	80,000 r	-
8	control (drumsticks) ^b	--	+

^aRefer to footnote "a" of Table 1.

^bControl consisted of whole carcass or drumstick that was artificially contaminated with the test organism and was not pasteurized but left at room temperature (27°C) for the same length of time as X-ray pasteurized drumsticks or whole carcass under irradiation.

Salmonella is very sensitive to irradiation as compared to other pathogens. The carcasses in this study were packaged similar to that of a commercial processing plant and were irradiated in cryovac bags. Results should have commercial significance, but owing to the rather limited sizes of the irradiation sources, the number of turkey drumsticks and whole carcasses irradiated was small.

Microwave irradiation of turkey drumsticks and broiler carcasses

Effect of microwaves on the destruction of S. senftenberg 775W on turkey drumsticks is shown in Table 10. The test organism and other bacteria were destroyed in 120 sec with microwaves on drumsticks, as evidenced by no growth on BGSP plating media, whereas 90 sec did not cause total destruction of the test organism. The test organism and other bacteria were effectively destroyed by microwaves in 600 sec on the broiler carcass, as evidenced by no growth on BGSP plating media, whereas treatment periods of 450 sec or less did not cause total destruction of the test organism (Table 10a).

Microwave treatment resulted in partially cooked meat, thus affecting the flavor and stability of the meat. The deleterious effects on meat, caused by the partial cooking may not be accepted by the consumers. If partial cooking had no effect on the flavor, shelf life, and acceptability of meat by consumers then microwave could possibly be the cheapest and quickest means of pasteurizing meat.

TABLE 10. EFFECTS OF MICROWAVE IRRADIATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W.

Sample No.	Pasteurization time in microwave oven	Salmonella isolated ^a	Skin score ^b
1	9 min.	- 1	3
2	7 min.	- 1	3
3	5 min.	- 1	3
4	3 min.	- 1	3
5	150 sec.	- 1	3
6	120 sec.	- 1	3
7	90 sec.	+	3
8	60 sec.	+	2
9	30 sec.	+	1
10	control ^c	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cControl consisted of drumstick that was artificially contaminated with test organism and was not pasteurized and left at room temperature (27°C) for desired length of time and then analyzed for Salmonella.

¹Refer to footnote "1" of Table 1.

TABLE 10a. MICROWAVE IRRADIATION OF WHOLE BROILER CARCASS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W.

Sample No.	Pasteurization time in microwave oven (sec.)	Salmonella isolated ^a	Skin score ^b
1	150	+	2
2	210	+	3
3	270	+	3
4	450	+	3
5	450	+	3
6	600	- ¹	3
7	control ^c	+	1

^a Refer to footnote "a" of Table 1.

^b Refer to footnote "b" of Table 1.

^c Control consisted of whole broiler carcass that was artificially contaminated with test organism and was not pasteurized but left at room temperature (27°C) for desired length of time before analyzing for Salmonella.

¹ Refer to footnote "1" of Table 1.

Sodium chloride pasteurization of turkey drumsticks

Sodium chloride solutions proved to be very ineffective in eliminating *Salmonella* from artificially contaminated turkey drumsticks. As shown in Table 11, three treatments were indicated to have eliminated the test organism. Overall sodium chloride solutions cannot be shown as a good pasteurizing fluid because the results were incongruent with each other, i. e., six percent sodium chloride for 180 minutes did not kill the test organism, but 1% sodium chloride for 120 minutes and 6% for 120 minutes indicated destruction. The pasteurization was performed at $21 \pm 2^{\circ}\text{C}$ and the treatment did not change the physical appearance of meat or skin.

When turkey drumsticks and turkey tails were artificially contaminated with *Salmonella typhimurium*, a ten percent sodium chloride solution failed to eliminate the test organism at $21 \pm 2^{\circ}\text{C}$ in 240 and 540 minutes (Table 11a). The test organism was isolated from all the six tails and drumsticks samples. The treatment did not change the physical appearance of drumsticks.

The informal taste panel readily detected the superiority of taste and flavor of meat treated with 6% sodium chloride solution for three hours at 21°C over 3% sodium borate, 4% sodium carbonate, and water treated meat at the same temperature and period of time.

Sodium chloride is a source of chloride and sodium and is commonly used for preserving foods. When *Salmonella* is suspended

TABLE 11. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM CHLORIDE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Salmonella isolated ^a
	Sodium chloride as percent of the solution	Time (min.)	
1	6	120	-
2	control ^b	120	+
3	1	180	-
4	2	180	+
5	3	180	+
6	4	180	-
7	control ^b	180	+
8	6	180	+
9	6	180	+
10	6	180	+
11	6	180	+
12	control ^b	180	+

^aRefer to footnote "a" of Table 1.

^bThe control consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as sodium chloride pasteurized drumsticks.

TABLE 11a. PASTEURIZATION OF TURKEY PARTS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM CHLORIDE SOLUTIONS (10% NaCl, $21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Salmonella isolated ^a
	Turkey parts	Time (min.)	
1	tail	240	+
2	tail	240	+
3	tail	240	+
4	tail	240	+
5	control (tail) ^b	240	+
6	drumstick	540	+
7	drumstick	540	+
8	control (drumstick) ^b	540	+

^aThe test organism, Salmonella typhimurium was deemed present if positively identified according to the following criterion; H₂S positive, Group B positive (modified Kaufman White scheme) and typical biochemical reactions of Salmonella.

^bRefer to footnote "b" of Table 11.

in high concentrations of sodium chloride, a diffusion gradient causes plasmolysis after which the cells burst. Apparently a 10% sodium chloride solution did not create diffusion gradients strong enough to cause plasmolysis of the *Salmonella* cells. There is also the possibility that some cells entered deeply in cuts of the drumsticks and never came in contact of pasteurizing fluid. Higher concentrations were not used because higher sodium chloride concentration would not be palatable to most consumers.

Chlorine pasteurization of turkey drumsticks

Chlorine, a widely used bacteriocidal agent, proved ineffective in eliminating *S. senftenberg* 775W and *S. typhimurium* from turkey drumsticks. As shown in Table 12, concentrations of chlorine solution as high as 3400 PPM, did not eliminate the test organism on turkey drumsticks in 180 minutes at $21 \pm 2^{\circ}\text{C}$. Chlorine solutions of concentrations less than 2550 PPM did not change the physical appearance of meat or skin; whereas, concentrations of 2550 PPM or higher of chlorine did cause changes in physical appearance of the drumsticks.

A reduction of bacterial growth was evidenced in selenite cystine broth when contaminated drumsticks were treated with 3400 PPM of chlorine. Though no quantitative analysis was performed, the selenite cystine broth containing pasteurized drumsticks appeared yellow indicating less growth of total bacteria, compared to deep red color of the selenite cystine broth containing the control drumsticks, indicating a heavy growth of bacteria.

TABLE 12. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING CHLORINE SOLUTIONS (180 min. at $21 \pm 2^{\circ}\text{C}$).

Sample No.	Chlorine PPM of the solution	Salmonella isolated ^a	Skin score ^b
1	106	+	1
2	212	+	1
3	318	+	1
4	425	+	1
5	637	+	1
6	850	+	1
7	1062	+	1
8	1275	+	1
9	control ^c	+	1
10	control ^c	+	1
11	1487	+	1
12	1700	+	1
13	1913	+	1
14	2125	+	1
15	2550	+	2
16	2975	+	2
17	3400	+	2
18	control ^c	+	1
19	control ^c	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cThe control consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as chlorine pasteurized drumsticks.

When turkey drumsticks were artificially contaminated with S. typhimurium, solutions of 2125 to 3400, PPM of chlorine failed to eliminate the test organism from the drumsticks in 180 and 540 minutes at $21 \pm 2^{\circ}\text{C}$ (Table 13). Solutions of 1700 and 2125 PPM chlorine did not eliminate S. typhimurium from drumsticks even in 24 hours at $21 \pm 2^{\circ}\text{C}$, but fewer Salmonella colonies were observed on BGSP plating media, relative to BGSP plates streaked from drumsticks treated for less time (9 hours). Drumsticks appeared yellow, had a greasy film over them, and were soft. Fat globules were observed on the skin and on the surface of the pasteurizing solutions. Since chlorine is a powerful oxidizing agent, it may have oxidized the fat thus giving yellow color to the drumsticks.

Chlorine is a widely used bacteriocidal agent but on the basis of these data, the bacteriocidal activity of chlorine against Salmonella, in the presence of organic matter such as poultry meat, can be questioned seriously. It might be that few cells of the test organism entered deeply into the cuts or feather follicles of the drumsticks and never came in direct contact with the pasteurizing fluid. Wabeck et al. (38) reported the number of Salmonella artificially inoculated on chicken drumsticks was slightly reduced by 20 and 40 PPM chlorine solutions.

Dixon and Pooley (11) reported that 200 PPM chlorine would effectively kill Salmonella when fewer than 10^3 organisms had been

TABLE 13. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING CHLORINE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Salmonella isolated ^a	Skin score ^b
	Chlorine PPM of the solution	Time		
1	3400	180 min.	+	2
2	3400	180 min.	+	2
3	control ^c	180 min.	+	1
4	2125	9 hr.	+	3
5	2125	9 hr.	+	3
6	3400	9 hr.	+	3
7	3400	9 hr.	+	3
8	control ^c	9 hr.	+	1
9	1700	24 hr.	+	3
10	1700	24 hr.	+	3
11	2125	24 hr.	+	3
12	2125	24 hr.	+	3
13	control ^c	24 hr.	+	1

^aRefer to footnote "a" of Table 11a.

^bRefer to footnote "b" of Table 1.

^cRefer to footnote "c" of Table 12.

distributed over the inside of a carcass in a fecal suspension. Heavily infected carcasses, such as those harboring 10^5 or more Salmonella are not rendered free from Salmonella by 200 PPM chlorine. Nillson and Regner (31) showed that chlorine concentration of 20 PPM reduced the surface concentration of the Salmonella (10^3 per square cm). Heavy inoculums (8×10^3 to 8×10^5 viable cells per ml of fluid) were used in the experiment reported herein. Obviously, chlorine was not effective against high concentrations of cells in the presence of poultry meat.

The strong possibility of chlorine evaporation from the treatment solution cannot be overruled. Ranken et al. (32) reported that with initial nominal concentration of free chlorine up to 200 PPM, little or none remained after four hours. In the experiment reported herein, sodium hypochlorite was used as a source of chlorine, which is relatively more stable than the gaseous type of chlorine. No experiments were performed on the extent of chlorine evaporation from the pasteurizing fluids.

Dixon and Pooley (11) reported that a higher dose of chlorine (more than 200 PPM) will change the flavor when meat is immersed in this concentration for more than 10 minutes. A higher concentration (3400 PPM) and longer immersion time were used in the experiment reported herein. On the basis of Dixon and Pooley's report, 3400 PPM chlorine would have definitely changed the flavor of the meat.

Chlorine odor was noticed in the room where pasteurization was performed, also on the pasteurized drumsticks. Chlorine should not be used as a pasteurizing fluid because it does not eliminate *Salmonella* from drumsticks and changes the flavor and physical appearance of the meat.

Sodium carbonate pasteurization of turkey drumsticks

The effect of sodium carbonate on the destruction of *Salmonella senftenberg* 775W on turkey drumsticks is shown in Table 14. One and two percent solutions of sodium carbonate did not eliminate the test organism in 90 minutes at 32°C, whereas a four percent solution of sodium carbonate reduced the test organism to an undetected level at the same temperature and time. *Salmonella* could not be isolated when pasteurization was performed at 88°C in one and two percent solutions of sodium carbonate irrespective of time (15 to 210 sec). The pasteurization at higher temperature (88°C) produced partially cooked drumsticks, probably due to high heat of the solution rather than concentration of the sodium carbonate.

Salmonella could not be isolated from a broiler carcass, when pasteurized with four percent sodium carbonate solution at 27°C for 120 minutes. However when the sodium carbonate was dissolved in a lightweight aluminum coated pan, a chemical reaction occurred which removed the coating of the pan. The chemical reaction of sodium carbonate with the aluminum may have helped in the elimination of the

TABLE 14. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM CARBONATE SOLUTIONS.

Sample No.	Pasteurization				
	Sodium Carbonate as percent of the solution	Temperature (°C)	Time	Salmonella isolated ^a	Skin score ^b
1	1	32	90 min.	+	1
2	2	32	90 min.	+	1
3	4	32	90 min.	-	1
4	control ^c	32	90 min.	+	1
5	1	88	15 sec.	-	2
6	1	88	30 sec.	-	2
7	2	88	210 sec.	-	3
8	control ^c	88	15 sec.	+	2
*9	4	27	120 min.	-	1
*10	control ^c	27	120 min.	+	1
11	0.5	49	60 sec.	+	1
12	1.0	49	60 sec.	+	1
13	1.5	49	60 sec.	+	1
14	2.0	49	60 sec.	-	1
15	control ^c	49	60 sec.	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cThe control consisted of drumsticks or whole broiler carcass that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and times as sodium carbonate pasteurized drumsticks or whole broiler carcass.

*Whole broiler carcass.

test organism from the broiler carcass. The treatment did not change the physical appearance of the broiler carcass.

A 0.5 to 1.5 percent solution of sodium carbonate failed to eliminate the test organism from drumsticks at 49°C in 60 sec, whereas a two percent solution of sodium carbonate eliminated the test organism under the same conditions. At 65°C , a one percent solution of sodium carbonate failed to eliminate the test organism from drumsticks in 60 sec, whereas 1.5 and 2% solutions of sodium carbonate eliminated the test organism under the same conditions. The 65°C treatment resulted in partially cooked drumsticks (Table 14a).

Sodium carbonate proved ineffective in eliminating S. senftenberg 775W at $21 \pm 2^{\circ}\text{C}$. The test organism was isolated from two of the four drumsticks treated with 5% sodium carbonate for 240 minutes at $21 \pm 2^{\circ}\text{C}$. Increased immersion time (540 minutes) or increased concentration of sodium carbonate did not help in eliminating the test organism at this temperature (Table 14a).

When turkey drumsticks were artificially contaminated with S. typhimurium, five and eight percent solutions of sodium carbonate failed to eliminate the test organism at $21 \pm 2^{\circ}\text{C}$ in four hours (Table 14b). The treatment did not affect the physical appearance of the drumsticks.

Pasteurization of the drumsticks at lower temperatures ($21 \pm 2^{\circ}$ or 32°C) did not alter the physical appearance of meat or skin. In

TABLE 14a. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM CARBONATE SOLUTIONS.

Sample No.	Pasteurization		Time	Salmonella isolated ^a	Skin score ^b
	Sodium Carbonate as percent of the solution	Temperature (°C)			
1	0.5	65	60 sec.	+	2
2	1.0	65	60 sec.	+	2
3	1.5	65	60 sec.	-	2
4	2.0	65	60 sec.	-	2
5	control ^c	65	60 sec.	+	2
6	5	21±2	240 min.	+	1
7	5	21±2	240 min.	-	1
8	5	21±2	240 min.	+	1
9	5	21±2	240 min.	-	1
10	control ^c	21±2	240 min.	+	1
11	5	21±2	540 min.	+	1
12	5	21±2	540 min.	+	1
13	control ^c	21±2	540 min.	+	1
14	8	21±2	240 min.	+	1
15	8	21±2	240 min.	+	1
16	control ^c	21±2	240 min.	+	1

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 1.

^cRefer to footnote "c" of Table 14.

TABLE 14b. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM CARBONATE SOLUTIONS (4 hours, $21 \pm 2^{\circ}\text{C}$).

Sample No.	Sodium Carbonate as percent of the solution	Salmonella isolated ^a
1	5	+
2	5	+
3	control ^b	+
4	8	+
5	8	+
6	control ^b	+

^aRefer to footnote "a" of Table 11a.

^bRefer to footnote "c" of Table 14.

fact pasteurized drumsticks were whiter and more attractive than the control drumsticks. Sodium carbonate, being a detergent, may have removed dirt and fat globules, accounting for the attractive appearance.

Salmonella typhimurium was easier to isolate from the turkey parts than S. senftenberg 775W. Enrichment and differential media have been designed for the isolation of Salmonella from fecal material. S. senftenberg 775W, not a common fecal organism, then was probably not favored as S. typhimurium, one of the more commonly isolated fecal organisms.

An informal taste panel could not detect the difference between the meat treated with 4% sodium carbonate, 3% sodium borate, and water for three hours at $21 \pm 2^{\circ}\text{C}$. Sodium carbonate is a white, odorless powder of alkaline taste and is used in softening the water. It is edible and can be used as a pasteurizing fluid if pasteurization is performed at higher temperature (49°C or above).

Sodium borate pasteurization of turkey drumsticks

Sodium borate proved to be very effective in eliminating Salmonella from turkey drumsticks. A two percent sodium borate failed to destroy S. senftenberg 775W in three hours at $21 \pm 2^{\circ}\text{C}$ whereas test organisms could not be isolated from two of the three drumsticks treated with 3% sodium borate at $21 \pm 2^{\circ}\text{C}$, for three hours (Table 15). Salmonella was effectively destroyed in four of the four drumstick samples treated with 4% sodium borate solution at

TABLE 15. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM BORATE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$, 3 HOURS).

Sample No.	Sodium Borate as percent of the solution	Salmonella isolated ^a
1	1.0	+
2	2.0	+
3	3.0	-
4	3.0	+
5	3.0	-
6	control ^b	-
7	4.0	-
8	4.0	-
9	4.0	-
10	4.0	-
11	control ^b	+
*12	3.0	+
*13	5.0	+
*14	control ^b	+

^aRefer to footnote "a" of Table 1.

^bThe control consisted of drumsticks or whole broiler carcasses that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and times as sodium borate pasteurized drumsticks or whole broiler carcass.

*Whole broiler carcass.

21 \pm 2^oC for three hours. Both three and five percent sodium borate solutions failed to destroy the test organism on broiler carcasses at 21 \pm 2^oC in three hours. Most likely the test organism remained protected in body cavities, feather follicles, cuts, or other pockets in the carcasses and never came in direct contact with the sodium borate solution.

A five to eight percent sodium borate solution failed to eliminate S. senftenberg 775W from drumsticks at 21 \pm 2^oC in 240 minutes (Table 15a). These observations are inconsistent with the previous trials. The difference could be attributed to the different sizes of drumsticks or test organisms embedded deeply enough in the meat to prevent exposure to the pasteurizing solution. Although eight percent sodium borate solutions did not eliminate the test organism completely in three hours at 21 \pm 2^oC, total bacterial count was greatly reduced. Only 50 and 60 bacterial colonies were observed on BGSP plating media, whereas control plates were heavily loaded with bacterial colonies, including Salmonella, and could not be counted. A five percent solution of sodium borate for 540 minutes at 21 \pm 2^oC reduced the total bacterial counts including Salmonella, even more than the 8% solutions for 240 minutes from drumsticks.

When turkey parts (drumsticks and tails) were artificially contaminated with S. typhimurium, three and four percent solution of sodium borate failed to destroy the test organism in three hours at

TABLE 15a. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA SENFTENBERG 775W USING SODIUM BORATE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Salmonella isolated ^a	Colony count on BGSP plating media
	Sodium borate as percent of the solution	Time		
1	5	240 min.	+	TNC
2	5	240 min.	+	TNC
3	8	240 min.	+	60
4	8	240 min.	+	50
5	control ^b	240 min.	+	TNC
6	5	540 min.	+	27
7	5	540 min.	+	16
8	control ^b	540 min.	+	TNC

^aRefer to footnote "a" of Table 1.

^bRefer to footnote "b" of Table 15.

TNC = Too numerous to count.

$21 \pm 2^{\circ}\text{C}$ (Table 15b). Salmonella typhimurium was isolated from six of the six drumsticks treated with 5% borate at $21 \pm 2^{\circ}\text{C}$ for four hours. An eight percent solution of sodium borate, though, did not eliminate S. typhimurium from drumsticks completely in 4 hours at $21 \pm 2^{\circ}\text{C}$, but greatly reduced the bacterial counts on BGSP plating media.

It appeared from these data that S. typhimurium is less sensitive to sodium borate than S. senftenberg 775W. The total bacterial colony counts on BGSP, from S. senftenberg 775W contaminated drumsticks treated with 5% borate at $21 \pm 2^{\circ}\text{C}$ for 9 hours was 16 and 27, whereas under the same experimental conditions the heavily overgrown BGSP plates, streaked from S. typhimurium contaminated drumsticks had colonies too numerous to count (Table 15c).

Since S. typhimurium appeared to be more resistant to sodium borate than S. senftenberg 775W, S. typhimurium was used for further experimentation. The hypothesis being, if sodium borate can destroy a more resistant strain of Salmonella, it will also destroy the less resistant strain, like S. senftenberg 775W.

Total bacterial colony counts were progressively reduced with higher concentrations of sodium borate solutions (Table 15c). Sodium borate concentrations of 7, 9 and 10% for nine hours at $21 \pm 2^{\circ}\text{C}$ did not eliminate S. typhimurium completely from drumsticks but greatly reduced total bacterial counts including the test organism, whereas a 12% borate solution produced "sterile" drumsticks under the same

TABLE 15b. PASTEURIZATION OF TURKEY CARCASS PARTS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM BORATE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Time	Salmonella isolated ^a
	Turkey carcass parts	Sodium borate as percent of the solution		
1	Tail	4	3 hr.	+
2	Tail	4	3 hr.	+
3	Tail	4	3 hr.	+
4	Tail	4	3 hr.	+
5	Tail	4	3 hr.	+
6	Tail	control ^b	3 hr.	+
7	Drumstick	3	3 hr.	+
8	Drumstick	3	3 hr.	+
9	Drumstick	control ^b	3 hr.	+
10	Drumstick	5	4 hr.	+
11	Drumstick	5	4 hr.	+
12	Drumstick	5	4 hr.	+
13	Drumstick	5	4 hr.	+
14	Drumstick	5	4 hr.	+
15	Drumstick	5	4 hr.	+
16	Drumstick	control ^b	4 hr.	+

^aRefer to footnote "a" of Table 11a.

^bThe control consisted of drumsticks or tail that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as sodium borate pasteurized drumsticks or tails.

TABLE 15c. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM BORATE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Salmonella isolated ^a	Colony count on BGSP plating media
	Sodium borate as percent of the solution	Time		
1	8	4 hr.	+	182
2	8	4 hr.	+	124
3	control ^b	4 hr.	+	TNC
4	5	9 hr.	+	TNC
5	5	9 hr.	+	TNC
6	control ^b	9 hr.	+	TNC
7	7	9 hr.	+	259
8	7	9 hr.	+	91
9	9	9 hr.	+	2
10	9	9 hr.	+	12
11	control ^b	9 hr.	+	TNC
12	10	9 hr.	+	10
13	10	9 hr.	+	8
14	12	9 hr.	-	nil
15	12	9 hr.	-	nil
16	control ^b	9 hr.	+	TNC

^aRefer to footnote "a" of Table 11a.

^bControl consisted of drumsticks that were artificially contaminated with the test organism, then pasteurized in distilled water for the same temperature and time as sodium borate pasteurized drumsticks.

TNC = Too numerous to count.

experimental conditions as evidenced by no growth on BGSP plating media (Fig. 5).

A total reduction in colony counts on BGSP plates, streaked from the drumsticks, treated with 7% sodium borate solution for 9 hours at $21 \pm 2^{\circ}\text{C}$ is shown in Fig. 2. Only 91 and 259 total bacterial colonies were counted on these plates. A further reduction in total bacterial count was observed when drumsticks were treated with 9 and 10% sodium borate solutions for 9 hours at $21 \pm 2^{\circ}\text{C}$ (Fig. 4). Practically no change in the original selenite cystine color was observed when drumsticks treated with 9 and 10 percent sodium borate solution for 9 hours at $21 \pm 2^{\circ}\text{C}$, were incubated at 37°C for 24 hours. Deep red color of selenite cystine broth was observed in control drumsticks (Fig 3).

It was easier to isolate *Salmonella* from the red strip shown on drumsticks (Fig. 3). Since *S. typhimurium* and *S. senftenberg* 775W are motile organisms, they may have migrated from selenite cystine broth to the drumsticks to avoid diffusion into the solution. The red strip on the drumsticks probably illustrates the area most favorable for the organism to avoid diffusion. The organisms accumulated in large numbers in this area may have facilitated easy isolation.

A 3 to 9 percent solution of sodium borate failed to eliminate *Salmonella* from drumsticks at $21 \pm 2^{\circ}\text{C}$ in 24 hours. However, a reduction in total bacterial counts was observed (Table 15d). As the

TABLE 15d. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM BORATE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$, 24 HOURS).

Sample No.	Sodium borate as percent of the solution	Salmonella isolated ^a	Colony count on BGSP plating media
1	3	+	TNC
2	3	+	TNC
3	5	+	280
4	5	+	190
5	control ^b	+	TNC
6	7	+	215
7	7	+	185
8	9	+	50
9	9	+	21
10	control ^b	+	TNC

^aRefer to footnote "a" of Table 11a.

^bRefer to footnote "b" of Table 15c.

TNC = Too numerous to count.

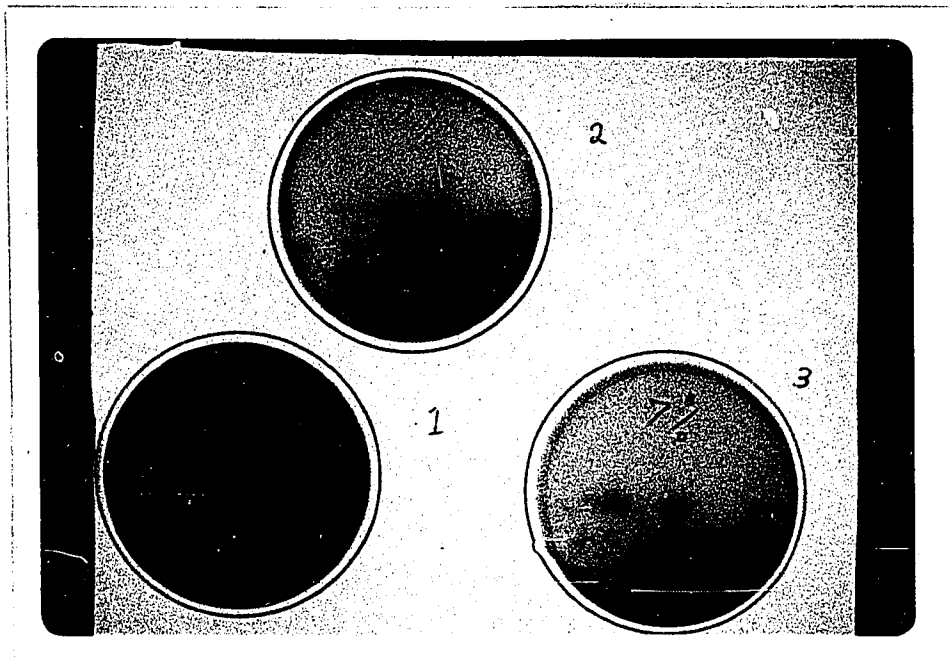


Figure II. BGSP agar plates illustrating difference between 7% sodium borate treated drumsticks and control drumsticks. 1. control; 2 and 3. treated with sodium borate.

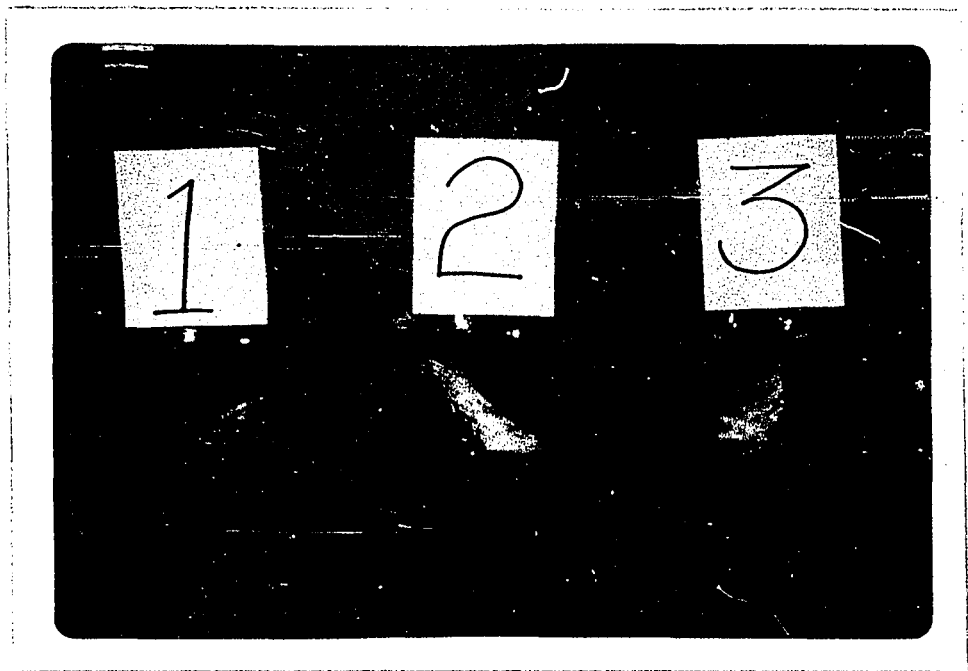


Figure III. Selenite cystine broth illustrating difference in bacterial growth between sodium borate treated drumsticks and control drumstick. 1. control, 2. treated with 9% sodium borate, 3. treated with 10% sodium borate.

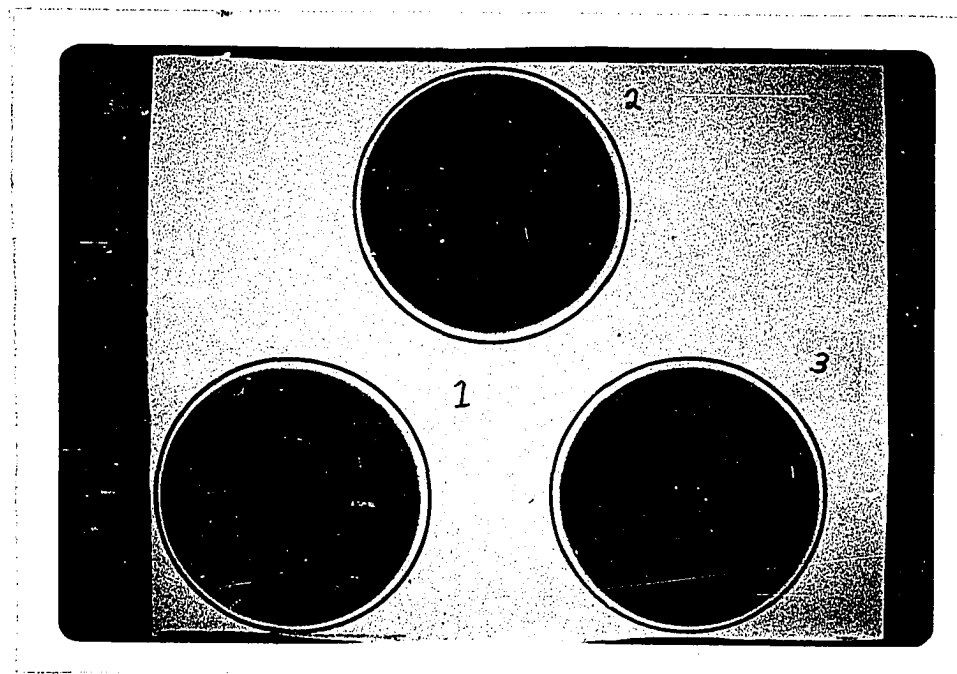


Figure IV. BGSP agar plates illustrating difference between sodium borate treated drumsticks and control drumstick.
1. control, 2. treated with 9% sodium borate, 3. treated with 10% sodium borate.

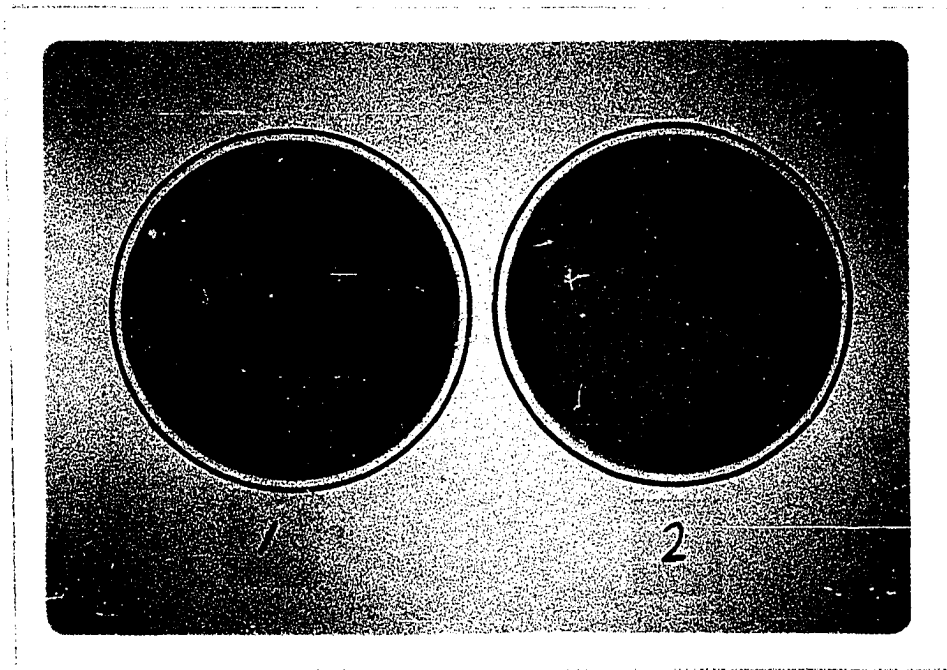


Figure V. BGSP agar plates illustrating difference between 12% sodium borate treated drumstick and control drumstick. 1. control, 2. treated with sodium borate.

concentration of the borate solution was increased, the total bacterial colony counts on BGSP plating media was decreased.

The effect of combined sodium borate and chlorine solution in the elimination of S. typhimurium is presented in Table 16. It was thought that the combination of ions, salts, and acids resulting from the mixed solution of sodium borate and chlorine might be more bacteriocidal than either compound alone. A 5% sodium borate and 200 PPM chlorine solution inconsistently eliminated the test organism at $21 \pm 2^{\circ}\text{C}$ during exposure period of 4, 6, and 8 hours. Salmonella was isolated from two of the four and one of the two drumsticks treated with this solution for six and eight hours. Salmonella was isolated from four of four tail samples treated with 5% borate and 200 PPM chlorine for eight hours at $21 \pm 2^{\circ}\text{C}$. Increased concentration of borate (6 and 7%) with 200 PPM chlorine did not aid in the elimination of the test organism from turkey tails.

No physical change in skin or meat color of the drumsticks was observed, irrespective of immersion time and concentration of borate solution. A thin layer of sodium borate was observed on the drumsticks when 8 to 12% borate solution was used irrespective of immersion time (4 to 24 hours). This layer can be washed with tap water which will reduce residual borax on the carcass.

Sodium borate pasteurized drumsticks were observed to be whiter and more attractive than the control drumsticks. Sodium borate is a powerful

TABLE 16. PASTEURIZATION OF TURKEY DRUMSTICKS CONTAMINATED WITH SALMONELLA TYPHIMURIUM USING SODIUM BORATE AND CHLORINE SOLUTIONS ($21 \pm 2^{\circ}\text{C}$).

Sample No.	Pasteurization		Time (hr.)	Salmonella isolated ^a
	Turkey parts	Sodium borate as percent of the solution and chlorine PPM		
1	Drumstick	5.0 + 200 PPM	4	+
2	Drumstick	5.0 + 200 PPM	6	-
3	Drumstick	5.0 + 200 PPM	6	+
4	Drumstick	5.0 + 200 PPM	8	+
5	Drumstick	5.0 + 200 PPM	8	+
6	Drumstick	5.0 + 200 PPM	8	-
7	Drumstick	5.0 + 200 PPM	8	-
8	Drumstick	control ^b	8	+
9	Tail	5.0 + 200 PPM	8	+
10	Tail	5.0 + 200 PPM	8	+
11	Tail	5.0 + 200 PPM	8	+
12	Tail	5.0 + 200 PPM	8	+
13	Tail	control ^b	8	+
14	Tail	6.0 + 200 PPM	8	+
15	Tail	6.0 + 200 PPM	8	-
16	Tail	7.0 + 200 PPM	8	+
17	Tail	7.0 + 200 PPM	8	+

^aRefer to footnote "a" of Table 11a.

^bRefer to footnote "b" of Table 15c.

detergent and sanitizer, it apparently removed dirt and dissolved fat and other particles, producing brighter and more attractive drumsticks. An informal taste panel failed to differentiate between the meat treated with 3% sodium borate or 4% sodium carbonate or water for three hours at $21 \pm 2^{\circ}\text{C}$. Higher concentrations of sodium borate may alter the chemical composition of the meat and produce undesirable taste.

CHAPTER V

SUMMARY AND CONCLUSION

Solutions of lactic acid (3%) and acetic acid (4%) eliminated Salmonella senftenberg 775W from turkey drumsticks at 32° C in ninety minutes, but produced off color drumsticks. A 0.1 percent solution of lysozyme eliminated S. senftenberg 775W at 21 ± 2° C in 180 minutes without affecting carcass quality. Eighty thousand rads of X-ray eliminated the test organism from drumsticks but failed to eliminate it on whole turkey carcass.

Microwave irradiation destroyed S. senftenberg 775W and other bacteria on turkey drumstick and broiler carcass in 120 and 600 sec, but produced partially cooked carcass. Chlorine and sodium chloride solutions were proved ineffective in destroying the test organism on turkey drumsticks. Sodium carbonate gave inconsistent results in elimination of Salmonella from drumsticks. Potassium hydroxide was effective in destroying the test organism on drumsticks but produced red meat and skin.

A twelve percent sodium borate solution destroyed Salmonella and other bacteria on drumsticks in 540 minutes at 21 ± 2° C. Sodium borate treated drumsticks were observed to be whiter and more attractive.

The data support the technical feasibility of surface pasteurization of ready-to-cook poultry with sodium borate, X-irradiation, and lysozyme. Carcasses immersed in sodium borate or lysozyme solutions, or subjected to X-irradiation showed destruction of Salmonella and less bacterial growth than controls. Such treatments incorporated into commercial processing plant could be of significant value in controlling Salmonella and other organisms. The effect of sodium borate and irradiation on consumers and processors is worthy of investigation.

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